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COMPARATIVE FEATURES OF THE BIOLOGICAL DIVERSITY OF THE GUT MICROBIOTA IN PEOPLE, RECEIVING NUTRITION IN STANDARDIZED CONDITIONS OF ISOLATED COMMUNITY

Alekseev D. M. [a],
Ermolaev A. V. [a],
Lyamin A. V. [a],
Gorbachev D. O. [a]

[a] FSBEI HE «Samara State Medical University» of Ministry of Healthcare of Russian Federation, Samara, Russian Federation.

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СРАВНИТЕЛЬНЫЕ ХАРАКТЕРИСТИКИ БИОЛОГИЧЕСКОГО РАЗНООБРАЗИЯ МИКРОБИОТЫ КИШЕЧНИКА У ЛЮДЕЙ, ПОЛУЧАЮЩИХ ПИТАНИЕ В СТАНДАРТНЫХ УСЛОВИЯХ ИЗОЛЯЦИОННОГО СООБЩЕСТВА

Алексеев Д. В. [1],
Ермолаев А. В. [1],
Лямин А. В. [1],
Горбачев Д. О. [1]

[1] ФГБОУ ВО «Самарский государственный медицинский университет» Минздрава России, Самара, Российская Федерация.

Abstract

The issue of the human gut microbiota influence on various chronic diseases has been raised in the scientific literature for quite a long time. In this regard, the study of various means to correct the microbiota via dietary changes has becoming increasingly relevant. However, microbiological diversity can also be affected by many external factors, such as acute infections, immune system disorders, antibiotics intake or chronic stress. Considering these facts, a promising approach is to examine functional nutrition in an organized isolated community. The study included 120 males aged 18 to 22 years, who formed an organized strictly isolated community. The study was conducted in two stages: before the community formation and 9 months afterwards. Actual nutrition of the study participants was assessed using the 24-hour diet reproduction method, during which the nutrition models were emphasized at two stages. The microbiological study consisted in seeding feces samples on an expanded list of solid growth media under anaerobic conditions. To analyze intestinal microbiota diversity, a coefficient of constancy (C) was used, according to which the taxa of microorganisms were included in constant, additional or transient groups of colon luminal microbiota. During the study, three types of tendencies were identified: an increase in the constant microbiota group due to the transition of microorganisms from the transient or additive group; an increase in the additional microbiota group due to the transition of microorganisms from the constant or transient group; absence of changes regardless of the study stage. The first group included taxa such as Enterococcus spp., Klebsiella spp., Lactobacillus spp., Staphylococcus spp., Streptococcus spp., the second group included Bifidobacterium spp., Citrobacter spp., Aspergillus spp., Bacillus spp., Clostridium spp., Lactococcus spp., Micrococcus spp., Pseudomonas spp. Representatives of the third group (transient microbiota) became the most prevalent microbial community in our study. Bacteroides spp. and Enterobacter spp. had boundary values of the coefficient of constancy between the transient and additional microbiota. Therefore, our study demonstrated specific changes in intestinal microbiota diversity among subjects who formed an organized isolated community.

Keywords: gut microbiota, nutritive status, biological diversity, organized isolated community, nutrition, microbiome.

Резюме

В научной литературе уже довольно продолжительное время поднимается вопрос влияния кишечной микробиоты человека на различные хронические заболевания. В связи с этим, становится всё более актуальной и такая тема, как различные способы коррекции микробиоты с помощью изменений в рационе питания. Однако на микробиологическое разнообразие может оказывать влияние и множество сторонних факторов, таких как острые инфекции, нарушения в работе иммунной системы, прием антибиотиков или хронический стресс. Учитывая данные факты, перспективным видом исследования является изучение функционального питания в организованном коллективе закрытого типа. В исследование были включены 120 человек мужского пола в возрасте от 18 до 22 лет, которые сформировали организованный строго изолированный коллектив закрытого типа. Исследование было проведено в два этапа: до формирования коллектива и через 9 месяцев после его формирования. Была проведена оценка фактического питания участников исследования с помощью метода 24-часового воспроизведения рациона, в ходе чего установили модели питания участников исследования на двух этапах. Микробиологическое исследование заключалось в посеве образцов кала участников исследования на расширенный перечень плотных питательных сред в анаэробных условиях. Для анализа разнообразия кишечной микробиоты применяли коэффициент постоянства (С), при установлении которого таксоны микроорганизмов относили к постоянной, добавочной или случайной группам просветной микробиоты толстой кишки. В ходе исследования было выявлено три типа закономерностей: увеличение представителей группы постоянной микробиоты кишечника за счет перехода микроорганизмов из транзиторной или добавочной группы; увеличение представителей группы добавочной микробиоты кишечника за счет перехода микроорганизмов из постоянной или транзиторной группы; отсутствие изменений независимо от этапа исследования. К первой группе были отнесены такие таксоны как *Enterococcus* spp., *Klebsiella* spp., *Lactobacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., ко второй группе - *Bifidobacterium* spp., *Citrobacter* spp., *Aspergillus* spp., *Bacillus* spp., *Clostridium* spp., *Lactococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. Представители третьей группы (транзиторной микробиоты) стали самым обширным микробным сообществом в нашем исследовании, при этом *Bacteroides* spp. и *Enterobacter* spp. имели пограничные значения коэффициента постоянства между транзиторной и добавочной микробиотой. Таким образом, в нашем исследовании были продемонстрированы определенные изменения в разнообразии состава кишечной микробиоты у группы людей, сформировавших организованный коллектив закрытого типа.

Ключевые слова: кишечная микробиота, пищевой статус, биологическое разнообразие, организованный закрытый коллектив, питание, микробиом.

1 Introduction

For quite a long time, the scientific literature has been raising the issue of the influence of the human gut microbiota on various chronic diseases associated with both the gastrointestinal tract and other organ systems. A range of authors associate specific changes in the composition of the gastrointestinal microbiological communities with the emergence of alimentary diseases [2, 11].

In this regard, the study of various ways to correct the microbiota via dietary changes is becoming increasingly relevant. In fact, it has been proven that the intestinal flora can be modified significantly, for example, in case of predominance of easily digestible carbohydrates or animal fats in nutrition. Knowledge of such patterns allows to create promising methods of diet therapy for various diseases, the pathogenesis of which involves the microbiome [1, 6, 9].

Specific features of the lifestyle of the average modern individual (disruption of the rational nutrition principles, including regularity of nutrition, chronic stress, etc.) can lead to an incorrect assessment of the interrelation between diversity of intestinal microbiota and nutritional status of individuals [4, 10].

Taking into account these facts, a promising type of research, devoted to assessment of the connection of the intestinal flora biological diversity with nutritional features, is the study of functional nutrition in an organized community of an isolated type.

The aim of the study was to evaluate the effect of nutrition in an organized isolated community on the biological diversity of the luminal gut microbiota

2 Materials and methods

The study included 120 men (aged in a range from 18 to 22 years, with average age 18.4 ± 0.7) who did not suffer from acute infectious diseases during the last six months and did not have individual dietary restrictions. Individuals, included in the study, formed an organized closed-type community, strictly isolated from the external environment.

The questionnaire and microbiological parts of the study were conducted in two stages: before 120 individuals formed organized isolated community and 9 months after they formed an organized isolated community.

An assessment of the actual nutrition of the study participants was carried out, for which the 24-hour diet reproduction method was used. The obtained data was processed by the computer program "Nutri-prof" (software version No. 2.9, computer No. 2018616124 dated 05.23.2018).

Based on the results of the actual nutrition assessment, five nutrition models of the study participants were emphasized: "high-calorie" model, with a high consumption of dairy, egg, meat and flour products; "carbohydrate with a high content of complex carbohydrates" model (porridge, cereals, pasta, potato meals); "protein-carbohydrate" model, which is characterized by a specific feature of the high level of consumption of fish and dairy products, cereals and pasta; "vegetable" model, which is characterized by a high level of vegetables, nuts and legumes consumption; "high-carbohydrate" (a high level of consumption of various cereals, porridge, pasta, potatoes, sweets, confectionery).

During the analysis of the second stage of the study, changes in the qualitative features of nutrition models were emphasized due to modifications in the actual nutritional status of the individuals against the background of a limited choice of products. As a result, 4 nutrition models were identified at this stage of the study: "high-calorie" (similar to stage 1); "rational", which is characterized by moderate consumption of meat and fish products, cereals, porridge, pasta and vegetables; "vegetable" (similar to stage 1); "carbohydrate", which is characterized by an increased consumption of fruit and berry drinks, tea, nuts and legumes, cereals, porridge and pasta.

Samples of colon contents (feces) were collected from 120 individuals included in the study. The material was transported to a microbiological laboratory in a sterile container with a capacity of 60 ml.

Sample preparation, sowing and incubation were carried out in anoxic conditions, which were provided using the «Bactron 300-2» anaerobic chamber (Sheldon Manufacturing, USA). Sowing was carried out by evenly distributing the suspension with the biomaterial over the surface of the solid growth medium in accordance with OST 91500.11.0004-2003.

For cultivation of samples expanded list of artificial solid growth media was used. The samples were cultivated at 37 °C during 5 days.

To identify all microorganisms, the MALDI-ToF mass spectrometry was used on a Microflex LT (Bruker, Germany) with the method of direct and extended application with the addition of 70% formic acid.

The study was approved by the Ethical Committee on Bioethics of the Samara State Medical University of the Ministry of Health of the Russian Federation, Protocol No. 252 dated 09.07.2022. All study participants voluntarily signed an informed consent form approved by the ethics committee as part of the study protocol before being included in the study.

Statistical analysis of the obtained data was carried out using the StatTech program v.4.7.3 (Stattech LLC, Russia). The coefficient of constancy (C) was used to analyze the diversity of the intestinal microbiota and assess the share of individual taxa in its structure. Depending on the frequency of isolation of individual taxa, they were assigned to several groups of the luminal microbiota of the colon: constant – if the frequency of isolation of the genus was more than 50% of all observations; additional – if the frequency of isolation was in the range of 25-50%; transient (accidental) – if the taxon is isolated less than 25%.

The coefficient of constancy for the genera of microorganisms was calculated using a special formula: $C = p * 100/P$, where p is the number of observations of species from an individual genus, and P is the total number of observations. The coefficient of constancy for the genera of microorganisms was calculated using a special formula: $C = p * 100/P$, where p is the number of observations of species from a separate genus, and P is the total number of observations.

3 **Results**

At the first stage of the study, 68 unique genera and 211 unique species of microorganisms were identified among the study participants, and at the second stage – 112 genera and 329 species. After biological diversity assessment at different stages of the organized isolated community formation, it was found that the only taxon that can be considered as a part of constant microbiota at all stages of the study is *Escherichia* spp., isolated from 100.0% of the examined individuals.

Three types of tendencies were identified for the rest of the identified taxa: an increase in the constant intestinal microbiota due to the transition of microorganisms from the transient or additional group; an increase in the additional intestinal microbiota due to the transition of microorganisms from a constant or transient group; the absence of changes in the values of the coefficient of constancy regardless of the study stage.

The following taxa were included in the first group of mentioned tendencies: *Enterococcus* spp. (was isolated in 84.2% of all cases at the first stage of study and in 85,8% of all cases at the second stage of study), *Klebsiella* spp. (the incidence was 49.2% at the 1st stage of study and 55.0% at the 2nd stage of study), *Lactobacillus* spp. (61.7% at the 1st stage and 53.3% at the 2nd stage), *Staphylococcus* spp. (24.2% at the 1st stage and 65.0% at the 2nd stage), *Streptococcus* spp. (33.3% at the 1st stage and 53.3% at the 2nd stage).

The second group of tendencies included such taxa as *Bifidobacterium* spp. and *Citrobacter* spp. with a frequency of isolation from 32.5% to 43.3% at the 1st stage of the study. At the 2nd stage, there was a tendency to increase in the coefficient of constancy to 48.3% in *Bifidobacterium* spp. and a reverse trend to decrease in the coefficient to 25.8% in *Citrobacter* spp. Nevertheless, these microorganisms remained within the conditional values of the additional microbiota. *Aspergillus* spp. with a coefficient of 52.5% at the 1st stage of the study, being in the group of constant microbiota, passes into the group of additional microbiota at the 2nd stage of study (44.2%). *Bacillus* spp., *Clostridium* spp., *Lactococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. at the 1st stage of the study vary in the prevalence from 10.0% to 20.8%, being a part of transient microbiota. However, at the 2nd stage of study, these values increase to the range 25.0%-35.0%, transferring these microorganisms to the additional group.

The third group included taxa that are part of the transient microbiota and their coefficient has not changed sufficiently during both stages of the study. The following taxa belong to this group: *Bacteroides* spp. (at the 1st stage it was isolated from 24.2% of the examined individuals and from 20.8% at the 2nd stage), *Brevibacterium* spp. (10.0% at the 1st stage and 5.0% at the 2nd stage), *Candida* spp. (3.3% at the 1st stage and 12.5% at the 2nd stage), *Comamonas* spp. (5.8% at the 1st stage and 4.2% at the 2nd stage), *Corynebacterium* spp. (12.5% at the 1st stage and 15.8% at the 2nd stage), *Enterobacter* spp. (10.8% at the 1st stage and 20.0% at the 2nd stage), *Kocuria* spp. (2.5% at the 1st stage and 14.2% at the 2nd stage), *Morganella* spp. (4.2% at the 1st stage and 3.3% at the 2nd stage), *Mycobacterium* spp. (5.8% at the 1st stage and 7.5% at the 2nd stage), *Nannizzia* spp. (4.2% at the 1st stage and 5.0% at the 2nd stage), *Parabacteroides* spp. (10.0% at the 1st stage and 14.2% at the 2nd stage), *Proteus* spp. (7.5% at the 1st stage and 14.2% at the 2nd stage), *Providencia* spp. (at the 2nd stage it was not isolated from the examined individuals, but at the 1st stage the incidence was 5.8%), *Pseudarthrobacter* spp. (2.5% at 1st stage and 4.2% at the 2nd stage), *Pseudoglutamicibacter* spp. (1.7% at the 1st stage and 4.2% at the 2nd stage), *Raoultella* spp. (10.8% at the 1st stage and 0.8% at the 2nd stage), *Salmonella* spp. (0.8% at the 1st stage and 3.3% at the 2nd stage), *Streptomyces* spp. (1.7% at the 1st stage and 11.7% at the 2nd stage), *Weissella* spp. (11.7% at the 1st stage and 14.2% at the 2nd stage).

It is worth mentioning that the transient intestinal microbiota has a persistent tendency to increase in the coefficient of constancy. Representatives of *Bacteroides* spp. and *Enterobacter* spp. have boundary values of the coefficient between the transient and additional groups. The transient microbiota is the most prevalent microbial community in our study.

4 **Discussion**

An organized isolated community is a promising model for research of various types. In the scientific literature it is possible to find cohort studies of patients that are functionally close to the concept of an organized community – for example, military personnel [8].

Studies of the intestinal microbiota composition in militaries, forming an organized group of an isolated type, were also conducted by other authors. In one of these studies, military personnel, who received a strictly controlled diet, had a reduced representation of lactic acid bacteria and an increased isolation frequency of *Veillonella* spp. and *Clostridium* spp. [5]. It should be noted that results of our study were less unambiguous. *Lactobacillus* spp. became less common at the 2nd stage of the study, although it remained as a part of constant microbiota, while the *Lactococcus* spp., on the contrary, was transferred from a transient to an additional microbiota.

The interrelation of individual taxa with specific types of nutritional status was also demonstrated in other works. For example, when easily digestible carbohydrates predominate in the diet, the diversity of the *Verrucomicrobia* decreases [3].

Nevertheless, there are certain contradictions in the design aspect of such experiments. Some authors report that while studying the relationship between nutritional status and the diversity of the intestinal microbiota in militaries, it is still difficult to exclude the influence of environmental stress factors, despite the regulated and controllable diet [5]. Moreover, there is certain evidence of the possible effects of physiological stress, resulting from increased physical activity, on microbiological diversity [7]. Consequently, when conducting such studies, it is necessary to take into account the specifics of certain types of organized isolated communities.

5 Conclusion

Therefore, our study demonstrated certain changes in the diversity of the intestinal microbiota in a group of people who formed an organized isolated community. It is necessary to conduct further study of other similar groups capable of forming such community with a minimum range of external stress factors, in order to study the effect of nutrition behavior on the microbiota in more detail, as well as to assess the significance of these interrelations for human health.

ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

Блок 1. Информация об авторе ответственном за переписку

Alekseev Dmitriy Vladimirovich – specialist of the Laboratory of Culturomic and Proteomic Research in Microbiology of Professional Center for Education and Research in Genetic and Laboratory Technologies;

address: 443079, Gagarina st. 18A, Samara, Russian Federation;

telephone: 8(917)124-85-10;

ORCID: 0000-0002-8864-4956;

e-mail: d.v.alekseev@samsmu.ru

Алексеев Дмитрий Владимирович – специалист лаборатории культуромных и протеомных исследований в микробиологии Научно-образовательного профессионального центра генетических и лабораторных технологий;
телефон: 8(917)124-85-10;

ORCID: 0000-0002-8864-4956;

e-mail: d.v.alekseev@samsmu.ru

Блок 2. Информация об авторах

Ermolaev Alexander Vadimovich – Assistant of the Department of General Hygiene;

ORCID: 0000-0003-4044-9139;

e-mail: a.v.ermolaev@samsmu.ru

Ермолаев Александр Вадимович – ассистент кафедры общей гигиены;

ORCID: 0000-0003-4044-9139;

e-mail: a.v.ermolaev@samsmu.ru

Lyamin Artem Viktorovich – Doctor of Medical Sciences, Associate Professor, Director of Professional Center for Education and Research in Genetic and Laboratory Technologies;

ORCID: 0000-0002-5905-1895;

e-mail:a.v.lyamin@samsmu.ru

Лямин Артём Викторович - д.м.н., доцент, директор Научно-образовательного профессионального центра генетических и лабораторных технологий;

ORCID: 0000-0002-5905-1895;

e-mail: a.v.lyamin@samsmu.ru

Gorbachev Dmitry Olegovich – Doctor of Medical Sciences, Associate Professor, Head of the Department of General Hygiene;

ORCID: 0000-0002-8044-9806;

e-mail:d.o.gorbachev@samsmu.ru

Горбачев Дмитрий Олегович – д.м.н., доцент, заведующий кафедрой общей гигиены;

ORCID: 0000-0002-8044-9806;

e-mail:d.o.gorbachev@samsmu.ru

Блок 3. Метаданные статьи

COMPARATIVE FEATURES OF THE BIOLOGICAL DIVERSITY OF THE GUT MICROBIOTA IN PEOPLE, RECEIVING NUTRITION IN STANDARDIZED CONDITIONS OF ISOLATED COMMUNITY

СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА БИОЛОГИЧЕСКОГО РАЗНООБРАЗИЯ КИШЕЧНОЙ МИКРОБИОТЫ У ЛЮДЕЙ, ПИТАЮЩИХСЯ В СТАНДАРТИЗИРОВАННЫХ УСЛОВИЯХ ЗАКРЫТОГО КОЛЛЕКТИВА

Сокращенное название статьи для верхнего колонтитула:

КИШЕЧНАЯ МИКРОБИОТА В КОЛЛЕКТИВЕ ЗАКРЫТОГО ТИПА

GUT MICROBIOTA IN ISOLATED COMMUNITY

Keywords: gut microbiota, nutritive status, biological diversity, organized isolated community, nutrition, microbiome.

Ключевые слова: кишечная микробиота, пищевой статус, биологическое разнообразие, организованный закрытый коллектив, питание, микробиом.

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